Effectiveness of biomaterial-based combination strategies for spinal cord repair – a systematic review and meta-analysis of preclinical literature

Alba Guijarro-Belmar[1,4]*, Anna Varone[1] *, Martin Rugema Baltzer[1], Saurav Kataria[1], Ezgi Tanriver-Ayder[2], Ralf Watzlawick[5], Emily Sena[2], Catriona J. Cunningham[1], Ann M. Rajnicek[1], Malcolm MacLeod[2], Wenlong Huang[1†], Gillian L. Currie[2#], Sarah K. McCann[2,3#]

[1]School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

[2]Centre for Clinical Brain Sciences, University of Edinburgh, Edinburgh, UK

[3]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, QUEST Center, Charitéplatz 1, 10117 Berlin, Germany

[4]Sainsbury Wellcome Centre, University College London, London, W1T 4JG, United Kingdom

[5]Department of Neurosurgery, Freiburg University Medical Center, Freiburg, Germany

*These authors contributed equally to this work

#Joint last authors

[†]Corresponding author

Corresponding author contact:

Dr Wenlong Huang

Email: w.huang@abdn.ac.uk

Phone: +44 1224 437290

Abstract

Study Design: Systematic review and meta-analysis of preclinical literature.

Objectives: To assess the effects of biomaterial-based combination (BMC) strategies for the treatment of Spinal Cord Injury (SCI), the effects of individual biomaterials in the context of BMC strategies, and the factors influencing their efficacy. To assess the effects of different preclinical testing paradigms in BMC strategies.

Methods: We performed a systematic literature search of Embase, Web of Science and PubMed. All controlled preclinical studies describing an *in vivo* or *in vitro* model of SCI that tested a biomaterial in combination with at least one other regenerative strategy (cells, drugs, or both) were included. Two review authors conducted the study selection independently, extracted study characteristics independently and assessed study quality using a modified CAMARADES checklist. Effect size measures were combined using random-effects models and heterogeneity was explored using meta-regression with tau^2, I^2 and R^2 statistics. We tested for small-study effects using funnel plot–based methods.

Results: 134 publications were included, testing over 100 different BMC strategies. Overall, treatment with BMC therapies improved locomotor recovery by 25.3% (95% CI, 20.3-30.3; n=102) and *in vivo* axonal regeneration by 1.6SD (95% CI 1.2-2SD; n=117) in comparison with injury only controls.

Conclusion: BMC strategies improve locomotor outcomes after experimental SCI. Our comprehensive study highlights gaps in current knowledge and provides a foundation for the design of future experiments.

Introduction

The inability of adult mammalian Central Nervous System (CNS) neurons to regrow in response to spinal cord injury (SCI) is due to their limited intrinsic regrowth capacity and a hostile post-injury environment [1]. The majority of preclinical SCI repair approaches have been monotherapies, including different pharmacological interventions such as neurotrophic and angiogenic factors, cell therapies, and rehabilitative training [2].

Neurotrophic factors are a heterogeneous group of molecules involved in the development of the CNS and they promote robust neuronal survival and neurite outgrowth in the developing and adult CNS [3]. Early phase clinical trials have tested the efficacy of neurotrophins using gene therapy in patients with neurodegenerative diseases and SCI [4, 5]. One limitation of neurotrophins is that they selectively stimulate the outgrowth of subpopulations of neurons; for example, brain-derived neurotrophic factor (BDNF) promotes axonal regrowth of sensory but not corticospinal neurons [3]. Therefore, multiple trophic factors should be combined for a spinal cord repair therapy and their types and doses should be chosen and optimised carefully [3]. Recently, angiogenesis has been appreciated as a key component of any CNS regenerative strategy because without new blood vessel formation waste products cannot be removed from the injury site and nutrients cannot be provided. Consequently, angiogenic factors such as vascular endothelial growth factor (VEGF) have been used to promote vascularization after SCI [6]. Furthermore, cell therapy is an attractive therapeutic approach for SCI as it can provide significant neuroprotection, recovery through cell replacement, trophic support, and immune modulation [7]. Despite these advantages there are still several challenges such as choice of cell type, cell harvesting and cell differentiation that impede translation of this therapy to the clinic [8]. Studies have suggested that neural stem cells (NSCs) and mesenchymal stem cells (MSCs) exert a clear therapeutic benefit. NSCs can differentiate into neurons or glial cells but autologous NSC transplantation is not readily feasible [9]. MSCs are a more appealing choice because of the ease for autologous transplantation and efficient expansion, yet their utility is confined to immunomodulatory and trophic effects and their neuronal differentiation is questioned [8]. Hence, fundamental questions regarding cell treatments still need to be answered.

However, given the pathophysiological complexity of SCI, any single intervention is unlikely to improve patient outcomes [10]. Instead, combination therapies seem necessary and among these, many are biomaterial-based [11]. Historically, biomaterials for SCI repair have been used because of their ability to provide structural or active growth support to damaged axons. Moreover, biomaterials can act as a delivery platform for cells and therapeutic molecules, and a localised depot for sustained drug release [11, 12]. Ideally, biomaterials for SCI repair should support axonal growth with appropriate stiffness, biocompatibility, and degradability [13, 14]. Moreover, they should be modifiable according to the injury e.g., injectable hydrogels for irregular cavities seen with contusion SCI or implantable scaffolds for defined injuries such as those following laceration SCI (Figure 1) [13, 14]. They can be natural, synthetic or a mixture of both. Natural biomaterials are widely available and obtained from sources such as plants, animals and DNA. They contain very regular structures due to highly-controlled synthesis and normally exhibit better biocompatibility than synthetic biomaterials. However, owing to their natural origin, they often contain contaminating molecules [15]. Synthetic biomaterials can be easily modified to optimise their mechanical properties and to contain functional sequences for cell signalling. They are also more easily sterilised than natural materials, and their degradation pattern can be controlled [11, 16-18].

Narrative reviews have focused on preclinical research on biomaterials for SCI repair and we have conducted systematic reviews of single therapeutic strategies for traumatic SCI repair [11, 14, 19-21]. However, no systematic and quantitative summary of biomaterial-based preclinical research exists. Therefore, we conducted a systematic review and meta-analysis to assess the evidence for biomaterial-based combination strategies for SCI. Our pre-specified objectives were to assess: (1) the characteristics and effects of the biomaterial only (BMO), when tested in the context of combination strategies for SCI *in vitro* and *in vivo*; (2) effects of biomaterial-based combination (BMC) strategies for SCI tested *in vitro* and/or *in vivo* and the impact of study quality, study design and publication bias; and (3) whether biomaterial properties and prior *in vitro* testing have an impact on the effectiveness of BMC strategies *in vivo.*

Methods

The study protocol was pre-registered on the CAMARADES website[22] and protocol deviations are described in the supplementary materials. We searched PubMed, Embase and Web of Science on April 28th 2016, and again on May 1st 2018, before data analysis commenced. Titles and abstracts identified in the search were screened independently by two reviewers and discrepancies resolved through discussion. We included all controlled preclinical studies, either *in vitro* or *in vivo*, that provided quantitative outcomes and described a BMC strategy that included a non-biomaterial therapy such as cells or drugs. BMO outcomes were also included when they formed part of a study assessing a BMC strategy. For *in vivo* outcomes, the control was defined as SCI without any treatment. For *in vitro* outcomes, the control was defined as cell culture, with no treatment added.

Two independent reviewers extracted data, including graphical data, from the included studies, resolving any discrepancies (including $\geqslant$10% difference in extracted values) via discussion. We extracted study-specific characteristics including biomaterial type/name/structure; animal sex/weight/species; injury type/level; combination strategy, and type of experiment e.g., "*in vivo* only" or "*in vivo*, *in vitro* and biomaterial property". The primary outcomes were *in vivo* locomotor recovery and *in vitro* and *in vivo* axonal regeneration (not including axonal sprouting). Inclusion/exclusion criteria and primary and secondary outcomes are further described in the supplementary material.

We extracted group-level data for SCI with treatment, SCI without treatment (control), and uninjured (sham) groups. For each outcome we extracted the number of animals or samples, outcome mean, and the Standard Error of the Mean (SEM) or Standard Deviation (SD) in each group, the time of intervention and the assessment time. We extracted outcomes from individual components of the combination if reported, specifying each comparison as "effect of combination'', "effect of biomaterial", "effect of drug", or "effect of cells". Full names of abbreviated biomaterials, drugs and cells are described in the supplementary material.

We assessed study quality using a modified CAMARADES checklist [23] comprising evaluation of: randomisation, allocation concealment, blinding, sample size calculation, animal welfare compliance, potential conflicts of interest, and animal exclusions (e.g., deaths, surgical failure). For each comparison between a treatment and a control group, we calculated an effect size. For *in vivo* locomotor comparisons we calculated a normalised mean difference (NMD) [21, 24], presented as percentage improvement in the treatment *vs*. control group. For all other comparisons, we calculated a standardised mean difference (SMD), presented as improvement in outcome in the treatment *vs.* control group, in SD units. We pre-specified a minimum of 25 independent comparisons needed to perform meta-analysis on the primary and secondary outcomes. We combined effect size measures using random-effects models with restricted maximum likelihood (REML) estimate of between-study variance. the combination of heterogeneous studies In preclinical systematic reviews, means that often the meta-analytic pooled estimate of effect is less important than examining the sources of heterogeneity: identifying the factors contributing to between-study differences and what they can tell us about the efficacy of the intervention under different conditions. To assess heterogeneity, we used tau^2 (between-study variance), I^2 (percentage of variation attributable to between-study heterogeneity) and adjusted R^2 ($adjR^2$; proportion of between-study variance explained by the covariate). Using univariate meta-regression, we evaluated the impact of the study design variables we pre-defined in our protocol. These included the variables related to risks of bias and internal validity that we assessed using the modified CAMARADES checklist (referred to as study quality variables, listed above), in addition to the following study design variables: animal type and sex, type and level of injury, time of assessment and administration of analgesia. Where the number of comparisons was sufficient (10 independent comparisons per variable included in the model), we also used multivariable meta-regression. Each study design or study quality variable contained two or more levels (e.g., true, false, not reported). Where one level of a binary variable contained >90% of comparisons, we did not carry out meta-regression. Where comparisons were unbalanced in a variable with more than two levels, we grouped levels with <5 comparisons into an “Other” level. For combination strategies, variable levels were grouped based on the biomaterial used, e.g., studies using collagen-based biomaterials combined with other strategies were

grouped into the “collagen + combination” level. Meta-regression was conducted on datasets with grouped comparisons.

Holm-Bonferroni adjusted critical *p* values were used to adjust for the number of univariate meta-regression analyses per objective and dataset. We assessed the presence of small-study effects using funnel-plots, Egger’s regression, and trim-and-fill. Small- study effects describes the phenomenon where smaller studies are often associated with larger treatment effects, potentially due to publication bias. All statistical analyses were performed using Stata (Release 16; StataCorp LP, USA).

Results

We identified 2068 publications in the literature search (eFigure 1), of which 134 were included (eTable 1).

Objective 1: Characteristics and effects of biomaterials used in combination strategies

We first analysed biomaterial-specific outcomes, where BMO effects in SCI models were established independently of combination strategies. We identified 68 and 63 comparisons for locomotor recovery and *in vivo* axonal regeneration, respectively (Figure 1). As only 17 comparisons were identified for *in vitro* axonal regeneration, no further analysis was conducted. eTable 2 summarises 58 comparisons for secondary outcomes. BMO treatment improved locomotor recovery by 7.9% (95% confidence interval [CI] 4.9-11, $p<0.0001$, $Tau^2=83.6$, $I^2=90.4\%$, n=68) and *in vivo* axonal regeneration by 1.1SD (95% CI 0.7-1.5, $p<0.0001$, $Tau^2=1.4$, $I^2=77.3\%$, n=63). Significant heterogeneity was found but could not be explained by biomaterial type (locomotor recovery: $p=0.691$, $Tau^2=85.3$, $I^2=89.7\%$, $adjR^2=0\%$; eFigure 2A and *in vivo* axonal regeneration: $p=0.959$, $Tau^2=1.5$, $I^2=78.3\%$, $adjR^2=0\%$). For locomotor recovery outcomes, 57%, 24% and 19% of biomaterials were identified as natural, synthetic, or mixed, respectively (eFigure 2A). Biomaterial format had no effect on locomotor recovery ($p=0.610$, $Tau^2=89.4$, $I^2=88.4\%$, $adjR^2=0\%$, Table 1A). Scaffold was the most commonly used format (33.8% of comparisons) and conferred a 10.4% improvement (95% CI 5.2-15.6%; Table 1A) in locomotor recovery. This was followed by non-injected hydrogel (used in 27.9% of comparisons). Thirty-two individual biomaterials were assessed for their effects on locomotor recovery and 30 for *in vivo* axonal regeneration. Thirty-seven percent of locomotor recovery and 59% of *in vivo* axonal regeneration comparisons evaluated individual biomaterials that were tested in fewer than 5 experiments (grouped as "Other"; Table 1B, C). No significant relationships existed between the biomaterial used and locomotor recovery ($p=0.510$, $Tau^2=78.4$, $I^2=87.2\%$, $adjR^2=6.3\%$; Table 1B) or *in vivo* axonal regeneration ($p=0.245$, $Tau^2=1.4$, $I^2=76.7\%$, $adjR^2=0.41\%$; Table 1C). Multivariable meta-regression including biomaterial type and format was conducted but could not explain a significant proportion of the heterogeneity in locomotor recovery ($p=0.814$, $Tau^2=89.4$, $I^2=85.4\%$, $adjR^2=0\%$) or *in vivo* axonal regeneration ($p=0.256$, $Tau^2=1.4$, $I^2=76.5\%$, $adjR^2=0\%$; eTable 3). Most analyses contained insufficient data to draw definitive conclusions

about efficacy of and differences between biomaterials, regardless of type and format. eFigure 2B-D provides the effect sizes of all biomaterials, illustrating high within-group variability.

Objective 2: Biomaterial-based combination strategies tested *in vitro* and/or *in vivo*

The analyses for this objective included all data from studies testing BMC strategies, i.e. *in vitro* evaluation before *in vivo* testing and *in vivo* testing only. We identified 102 and 117 comparisons for locomotor recovery and *in vivo* axonal regeneration, respectively (Figure 2). As only 12 comparisons were identified for *in vitro* axonal regeneration, no further analysis was conducted. eTable 4 summarises 63 secondary outcomes. BMC treatments significantly enhanced locomotor recovery by 25.3% (95% CI 20.3-30.3%, $p<0.0001$, $Tau^2=543$, $I^2=98.4\%$, n=102), and *in vivo* axonal regeneration by 1.6SD (95% CI 1.2-2SD, $p<0.0001$, $Tau^2=2.5$, $I^2=86.3\%$, n=117). Treatment effects of different BMC strategies on behavioural and histological outcomes are detailed in eFigures 3A-C, 4A-C. Seventy-two combinations were assessed for their effect on locomotor recovery and 64 for *in vivo* axonal regeneration (eFigures 2-3). Outcomes were grouped according to biomaterial for meta-regression (Table 2) but we did not find significant effects of combinations (locomotor recovery: p=0.142, $Tau^2=520.7$, $I^2=97.7\%$, n=102; *in vivo* axonal regeneration: p=0.124, $Tau^2=2.2$, $I^2=84.1\%$, n=117). Poly(lactic-co-glycolic acid)(PLGA)-based and chitosan-based combinations had large effects on *in vivo* axonal regeneration [2.8SD (95% CI 0.7-4.8SD) and 2.9SD (95% CI 0.9-4.8SD); Table 2B] compared to control. PGLA-based combinations also had a large effect on locomotor recovery [41.5% (95% CI 16.7-66.3%); Table 2A]. Most other combinations, grouped according to biomaterial, had no measurable effects on *in vivo* axonal regeneration (Table 2B).

We next investigated the effect of seven study quality items on locomotor recovery outcomes (Figure 3A; eFigure 5). Blinding and randomisation were reported in 83.3% and 45.1% of comparisons, respectively. Few studies provided a description of the randomisation method (n=20/86). Only 47.1% and 28.4% of comparisons provided conflict of interest statements and animal exclusions, respectively. Allocation concealment and sample size calculation were rarely reported (4.9% and 1.0%, respectively; Figure 3A). The

average animal numbers per group was 10.6±12.1 for control and 10.9±12.4 for treatment. No quality measure was significantly associated with locomotor recovery (eFigure 5A-D).

SCI level of injury was a significant source of heterogeneity (p=0.008, Tau^2=488.1, I^2=97.8%, n=102; eFigure 6D). Mid-thoracic level SCI injury was most commonly used and accounted for 85% of comparisons. In these models treatment improved locomotor recovery by 26.8% (95% CI 21.7-32). Eleven percent of comparisons involved cervical level injury, however in these models treatment had no significant effect on locomotor recovery (6.4% improvement; 95% CI -8.9-21.7). The variables sex, post-surgical analgesia, and SCI type (contusion, compression, transection and hemisection) were not significant sources of heterogeneity (eFigure 6A-D). The most common last assessment time points were 8 (24%) and 4 weeks (18%) post-SCI. Transection and hemisection SCIs had the highest frequency (44% and 38%, respectively). For species and sex, rodents and females as animal models accounted for 95% and 70% of comparisons, respectively ; no differences were found in locomotor recovery between sexes.

The majority of included studies (98%) administered the BMC treatment acutely, straight after injury or briefly after it (0-7 days after injury), with only 2% of studies applying the treatment in a subacute manner (14 or more days after injury).

No evidence of small-study effects was found using Egger's regression test. Furthermore, with trim-and-fill analysis we did not detect any theoretically missing studies.

Objective 3: Effects of biomaterial-based combinations tested *in vivo* only *vs.* a full testing paradigm

Finally, we sought to determine whether prior *in vitro* assessments of biomaterial characteristics were associated with a greater improvement in *in vivo* outcomes after BMC treatment. "*In vivo* testing only" refers

to studies where the authors did not present or reference previous *in vitro* work characterising biomaterials as part of the rationale for their *in vivo* experiments testing BMC strategies. The effects of BMC strategies that underwent "*in vivo* testing only" were assessed in 47/103 *in vivo* locomotor recovery outcomes and 46/117 *in vivo* axonal regeneration outcomes from Objective 2 (Figure 2). Overall, BMC strategies tested only *in vivo* significantly improved locomotor recovery by 25.3% (95% CI 18.3-32.3%, $p<0.0001$, $Tau^2=466.3$ and $I^2=97.8\%$, n=47) and axonal regeneration by 1.3SD (0.7-2.1SD, $p<0.0001$, $Tau^2=2.3$, $I^2=88.1\%$, n=46). The specific BMC treatment (grouped according to biomaterial; Table 3) was a significant source of heterogeneity (locomotor recovery: $p=0.006$, $Tau^2=318.4$, $I^2=95.9\%$, n=47). PLGA-based combinations showed the greatest improvement in locomotor recovery (46.3%, 95% CI 26.4-66.3%, p=0.002; Table 3A). Treatment effects of different BMC strategies on behavioural and histological outcomes are detailed in eFigure 7.

A testing paradigm where BMO properties were assessed and the BMC was tested *in vitro,* prior to *in vivo* testing, did not result in a greater improvement in locomotor recovery (27.5%, 95% CI 18.3-36.8%, n=29; $p=0.696$, $Tau^2=526.2$, $I^2=97.72\%$ and $adjR^2=0\%$) than combinations that were tested *in vivo* only (25.2%, 95% CI 13.3-37.1, n=47; Figure 3B).

Lastly, we conducted *post-hoc* analysis of the influence of treatment type (BMO, individual therapies, or BMC) on locomotor recovery outcomes from all included studies. The type of treatment had a significant effect on locomotor recovery ($p<0.0001$, $Tau^2=351.7$, $I^2=97.5\%$, $adjR^2=14\%$; n=198; eFigure 8). Biomaterial-based combination treatments resulted in the greatest improvement in locomotor recovery compared to SCI only control [25.3% (95% CI 21.2-29.5); n=102]. This was followed by drugs alone [19.9% (95% CI 8.6-31.2); n=15], cells alone [12.8% (95% CI -0.1-25.8); n=13], and biomaterials alone [8.7% (95% CI 2.1-15.2); n=68] compared to SCI only control.

Discussion

Several reviews have identified and evaluated potential biomaterial-based therapies for SCI repair [14, 20, 25, 26] but none have described the impact of the biomaterial and biological and experimental design factors

on efficacy in a transparent summary of all available data. We have investigated three factors important in identifying promising BMC strategies for SCI: biomaterial properties, effectiveness of the combination strategy, and most effective preclinical testing paradigm.

Treatment with BMO resulted in a significant improvement in locomotor recovery and axonal regeneration. However, the specific biomaterial, biomaterial type and format could not explain the significant heterogeneity observed. This is likely due in part to the high number of different biomaterials used relative to the total number of studies: most individual biomaterials were tested in fewer than five comparisons. Additionally, there were low animal numbers reported per study, resulting in imprecise estimates of effect. Our analysis showed that natural biomaterials were used most frequently, likely explained by their excellent biocompatibility, mechanical and degradation properties, and ability to initiate neovascularisation [26]. Synthetic biomaterials were less commonly used but have exceptional properties, including high water content, mechanical stability, and ease of chemical modification to include integration of cell adhesion peptides [16, 17]. However, they are not easily cleared after degradation, which should be a focus area for future research [18]. It was interesting to note that hydrogels were the most frequently used biomaterial format. This technology offers the advantage of creating a complex and precise 3D geometry that conforms exactly with the lesion cavity [27]. Based on the diverse mechanical and biological features of different biomaterials, these factors are likely important determinants of success in combination strategies, and our results highlight an area where more research is needed to draw definitive conclusions regarding relative efficacy.

The SCI research community has reached a consensus that a combination therapeutic strategy is a necessity for SCI repair [26, 28]. However, this agreement was not supported by quantitative data. Our findings add weight to the consensus, by demonstrating that combination treatments improve locomotor recovery by 25.3% and *in vivo* axonal regeneration by 1.6SD. It appears that biomaterial-based combination strategies are more effective than cell- or drug-based single strategies. However, this finding should be interpreted

with caution, as we did not review all available evidence for these single strategies and this analysis was *post-hoc*.

Our findings support the potential of BMC approaches to tackle the physical and chemical barriers to SCI repair as well as the lack of intrinsic capacity of adult CNS neurons to regrow. The results of these BMC studies indicate that a biomaterial can be used not only as a permissive substrate to encourage injured axons to regrow but also as a delivery mechanism for cells and drugs. For example, Teng et al. implanted a polymer scaffold combined with NSC, which promoted functional recovery in an adult rat hemisection SCI model [29]. Overall, combinations based on PLGA resulted in robust improvements in outcomes. This US Food and Drug Administration (FDA)-approved synthetic biomaterial [30] has been studied in a variety of forms, including guidance channels, microsphere-loaded hydrogels and scaffolds [17, 31, 32], because of its excellent biocompatibility and degradability profile. Interestingly, PLGA was the biomaterial used in the only paper included in our review using non-human primates as a preclinical animal model [33]. The specific combination used in this study (PLGA and neural stem cells) was previously used in other studies using rats [29, 34], also included in this systematic review and meta-analysis. Interestingly, the only ongoing clinical trial with BMO for SCI repair uses Neuro-Spinal Scaffold™, which is a PLGA-based biomaterial [35]. However, no clinical trial using a biomaterial-based combination has been reported yet. Limitations to clinical translation would likely include regulatory obstacles such as the requirement by the FDA to show efficacy in human patients of not only the biomaterial alone but also the individual efficacy of any other non-biomaterial BMC components.

We did not find a significant difference in the efficacy of BMC strategies where biomaterial properties and *in vitro* efficacy were evaluated before *in vivo* experiments, compared to studies where only *in vivo* testing was carried out. This deserves further investigation when more researchers adopt the *prior* testing/evaluating approach. We advocate such an approach as it would ensure that unsuitable biomaterials do not move into *in vivo* testing for SCI repair, reducing research waste and contributing to more 3Rs (Replacement, Reduction and Refinement)-aligned research.

Preclinical SCI models have historically included rodents, cats, dogs and non-human primates. We found that rats were most commonly used in these studies, likely due to their small size, ease of handling, and the many well-developed, robust locomotor tests available to assess recovery [36]. Recently, the use of non-human primates in SCI repair research has received greater attention, especially to validate strategies with promise for clinical translation [37, 38]. However, the ethical concerns and financial challenges of using primates remain serious obstacles. We found that mid thoracic region injury was commonly used, and we suggest future research include more cervical models, as human SCI commonly occurs at the cervical region [39, 40]. We also observed that transection and hemisection were most frequently studied in animals, despite contusion being the most common injury type in humans. We understand that for biomaterial-based combination studies transection is more amenable at the early experimental stages, due to the less complex surgery, better postoperative recovery, and easier control of the cavity size. However, we suggest that subsequent testing also incorporate contusion models.

We found that steps to reduce the risk of bias were not a significant source of heterogeneity in the data. The prevalence of randomisation and blinding in our study is higher than that previously observed [21, 41], providing confidence in the findings reported here. We found few studies reported sample size calculations. This is a concern as too-small sample sizes can lead to imprecision and low reproducibility, while too-large sample sizes result in a waste of resources and excessive animal use [42]. We recommend the use of tools including the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) Experimental Design Assistant for preclinical study design [43].

Limitations

Our research question was broad, encompassing all *in vitro* and *in vivo* research on BMC strategies investigated for SCI repair. This approach, while providing a comprehensive overview of the field, has limited more specific conclusions. Importantly, *In vitro* models can only ever mimic certain aspects of SCI and what we infer from these experiments must be informed by an understanding of their biological and pathophysiological limitations. Moreover, there are currently no experimental validity standards for *in vitro*

295 models in SCI research. Our review provides a comprehensive overview of models used in the context of
296 biomaterial-related research that can contribute to generating such standards within the community.

297 In general, we found high variability between studies and a lack of data for many strategies that have not
298 been tested in a sufficient manner. This limited our ability to draw robust conclusions about the relative
299 efficacy of BMC strategies, and very little of the observed heterogeneity in the datasets was explained by the
300 variables investigated. However, it may be that unreported or unmeasurable variables contribute to this
301 heterogeneity e.g., noise level in the animal house or method of handling animals. Due to the high number
302 of different biomaterials and combinations studied, we grouped data for meta-regression based on the
303 biomaterial used. For combinations, we were therefore unable to examine potential differences in strategies
304 using cells, drugs or both. Even after grouping, 38% of comparisons evaluated BMC strategies that were
305 tested in fewer than five locomotor experiments.

306 A broader limitation of these approaches is their relatively low statistical power when the number of included
307 studies is modest [44]. Several outcomes were not analysed as the minimum number of required
308 comparisons was not reached. A general limitation of systematic review and meta-analysis is that these tools
309 can be used to summarise available evidence but cannot overcome deficiencies in quality, reporting or scope,
310 instead only highlighting where gaps in evidence exist. Further, these approaches cannot correct reporting
311 biases, including selective and incomplete reporting and publication bias [45]. In the studies included in the
312 current review, key experimental features were often not reported, including for what purpose a biomaterial
313 was synthesised or isolated, and what type of barrier(s) to neural repair and/or functional recovery it aimed
314 to overcome. This limited our ability to gain insights into the biological processes targeted by different
315 biomaterials and investigate which type(s) of biomaterials produced more reliable results in the context of
316 different injury models.

Conclusion

Our study provides a comprehensive summary of biomaterial-based combination strategies tested in preclinical SCI models. We demonstrate the effectiveness of these strategies overall for improving locomotor recovery and axonal regeneration. A diverse range of combination strategies has been tested and, while some appear more promising than others, a lack of evidence for many biomaterials and combinations limits our ability to draw definitive conclusions about their relative efficacy. Importantly, we highlight where gaps exist in our current knowledge and identify promising strategies to pursue in future preclinical research directed at SCI repair. Moving forward, it is important to note that the majority of included studies carried out implantations of biomaterials at an acute phase following SCI. It is imperative that researchers adopt appropriate *in vivo* models at sub-acute and chronic stages to assess biomaterial-based combination strategies at clinically relevant time points. Finally, biomaterial suitability for SCI repair should be assessed using *in vitro* and/or *ex vivo* models before advancing to *in vivo* testing, to minimise the likelihood of a major animal welfare concern.

(1343)

Data Archiving and Data Availability

The statistical analysis code and datasets analysed during this study are openly available from the Open Science Framework at https://osf.io/bgw73/.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

AGB and AV were responsible for designing the review protocol, screening potentially eligible studies, extracting and analysing data, conducting the meta-regression analyses, interpreting results, creating figures and tables, and writing the report. MRB and SK screened potentially eligible studies, extracted and analysed part of the data. ETA contributed to interpreting results. RW contributed to the design of the review protocol and provided feedback on the report. ES and MM contributed to the design of the review protocol,

interpreting results and writing the report. CJC and AMR contributed to writing the report and provided feedback. WH was responsible for designing the review protocol, interpreting results and writing the report. GLC and SKM were responsible for designing the review protocol, conducting the meta-regression analyses, interpreting results and writing the report

Funding

This work was supported by the Institute of Medical Sciences of the University of Aberdeen, International Spinal Research Trust, Scottish Rugby Union, RS McDonald Charitable Trust and The European Union's Horizon 2020 research and innovation programme (Marie Skłodowska-Curie grant agreement no. 702213).

References

1. Fitch MT, Silver J. CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. Exp Neurol. 2008;209(2):294-301. doi: 10.1016/j.expneurol.2007.05.014. PubMed PMID: WOS:000253184100002.
2. Fakhoury M. Spinal cord injury: Overview of experimental approaches used to restore locomotor activity. Reviews in the Neurosciences. 2015;26(4):397-405. doi: 10.1515/revneuro-2015-0001.
3. Lacroix S, Tuszynski MH. Neurotrophic factors and gene therapy in spinal cord injury. Neurorehabil Neural Repair. 2000;14(4):265-75. doi: 10.1177/154596830001400403. PubMed PMID: 11402877.
4. Silva NA, Sousa N, Reis RL, Salgado AJ. From basics to clinical: A comprehensive review on spinal cord injury. Prog Neurobiol. 2014;114:25-57. doi: 10.1016/j.pneurobio.2013.11.002. PubMed PMID: WOS:000335876800003.
5. Tuszynski MH. Growth-factor gene therapy for neurodegenerative disorders. Lancet Neurol. 2002;1(1):51-7. doi: 10.1016/s1474-4422(02)00006-6. PubMed PMID: WOS:000177694100020.
6. Sakiyama-Elbert S, Johnson PJ, Hodgetts SI, Plant GW, Harvey AR. Scaffolds to promote spinal cord regeneration. Handbook of clinical neurology. 2012;109:575-94. doi: 10.1016/b978-0-444-52137-8.00036-x. PubMed PMID: MEDLINE:23098738.
7. Badner A, Siddiqui AM, Fehlings MG. Spinal cord injuries: how could cell therapy help? Expert Opin Biol Ther. 2017;17(5):529-41. doi: 10.1080/14712598.2017.1308481. PubMed PMID: WOS:000399490700002.
8. Iyer NR, Wilems TS, Sakiyama-Elbert SE. Stem cells for spinal cord injury: Strategies to inform differentiation and transplantation. Biotechnol Bioeng. 2017;114(2):245-59. doi: 10.1002/bit.26074. PubMed PMID: WOS:000392539800001.
9. Raspa A, Pugliese R, Maleki M, Gelain F. Recent Therapeutic Approaches for Spinal Cord Injury. Biotechnol Bioeng. 2016;113(2):253-9. doi: 10.1002/bit.25689. PubMed PMID: WOS:000368188600001.
10. Sofroniew MV. Dissecting spinal cord regeneration. Nature. 2018;557(7705):343-50. Epub 2018/05/16. doi: 10.1038/s41586-018-0068-4. PubMed PMID: 29769671.
11. Haggerty AE, Oudega M. Biomaterials for spinal cord repair. Neurosci Bull. 2013;29(4):445-59. Epub 2013/07/18. doi: 10.1007/s12264-013-1362-7. PubMed PMID: 23864367; PubMed Central PMCID: PMCPMC5561944.
12. Pakulska MM, Ballios BG, Shoichet MS. Injectable hydrogels for central nervous system therapy. Biomed Mater. 2012;7(2):024101. Epub 2012/03/29. doi: 10.1088/1748-6041/7/2/024101. PubMed PMID: 22456684.

13. Varone A, Rajnicek AM, Huang W. Silkworm silk biomaterials for spinal cord repair: Promise for combinatorial therapies. Neural Regeneration Research. 2018;13(5):809-10. doi: 10.4103/1673-5374.232471.
14. Liu S, Schackel T, Weidner N, Puttagunta R. Biomaterial-Supported Cell Transplantation Treatments for Spinal Cord Injury: Challenges and Perspectives. Frontiers in Cellular Neuroscience. 2018;11(January). doi: 10.3389/fncel.2017.00430.
15. Buddy Ratner AH, Frederick Schoen, Jack Lemons. An Introduction to Materials in Medicine. 3rd Edition ed: Elsevier; 2013.
16. Ropper AE, Thakor DK, Han IB, Yu D, Zeng X, Anderson JE, et al. Defining recovery neurobiology of injured spinal cord by synthetic matrix-assisted hMSC implantation. Proc Natl Acad Sci U S A. 2017;114(5):E820-E9. doi: 10.1073/pnas.1616340114. PubMed PMID: WOS:000393196300020.
17. Pritchard CD, Slotkin JR, Yu D, Dai HN, Lawrence MS, Bronson RT, et al. Establishing a model spinal cord injury in the African green monkey for the preclinical evaluation of biodegradable polymer scaffolds seeded with human neural stem cells. Journal of Neuroscience Methods. 2010;188(2):258-69. doi: 10.1016/j.jneumeth.2010.02.019. PubMed PMID: WOS:000277866300011.
18. Trimaille T, Pertici V, Gigmes D. Recent advances in synthetic polymer based hydrogels for spinal cord repair. Comptes Rendus Chimie. 2016;19(1-2):157-66. doi: 10.1016/j.crci.2015.03.016.
19. Nomura H, Tator CH, Shoichet MS. Bioengineered Strategies for Spinal Cord Repair. Journal of Neurotrauma. 2006;23(3-4):496-507. doi: 10.1089/neu.2006.23.496.
20. Krishna V, Konakondla S, Nicholas J, Varma A, Kindy M, Wen X. Biomaterial-based interventions for neuronal regeneration and functional recovery in rodent model of spinal cord injury: a systematic review. J Spinal Cord Med. 2013;36(3):174-90. doi: 10.1179/2045772313Y.0000000095. PubMed PMID: 23809587; PubMed Central PMCID: PMCPMC3654443.
21. Antonic A, Sena ES, Lees JS, Wills TE, Skeers P, Batchelor PE, et al. Stem Cell Transplantation in Traumatic Spinal Cord Injury: A Systematic Review and Meta-Analysis of Animal Studies. PLoS Biol. 2013;11(12):14. doi: 10.1371/journal.pbio.1001738. PubMed PMID: WOS:000329367200003.
22. CAMARADES. CAMARADES Preclinical Systematic Review & Meta-analysis Facility (SyRF) 2021. Available from: http://syrf.org.uk/.
23. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias. Stroke. 2004;35(5):1203-8. Epub 2004/04/03. doi: 10.1161/01.STR.0000125719.25853.20. PubMed PMID: 15060322.
24. Vesterinen HM, Sena ES, Egan KJ, Hirst TC, Churolov L, Currie GL, et al. Meta-analysis of data from animal studies: A practical guide. Journal of Neuroscience Methods. 2014;221:92-102. doi: 10.1016/j.jneumeth.2013.09.010.
25. Tsintou M, Dalamagkas K, Seifalian AM. Advances in regenerative therapies for spinal cord injury: a biomaterials approach. Neural Regeneration Research. 2015;10(5):726-42. doi: 10.4103/1673-5374.156966. PubMed PMID: WOS:000355964400018.
26. Haggerty AE, Maldonado-Lasuncion I, Oudega M. Biomaterials for revascularization and immunomodulation after spinal cord injury. Biomed Mater. 2018;13(4):14. doi: 10.1088/1748-605X/aaa9d8. PubMed PMID: WOS:000430942600002.
27. Lee SJ, Esworthy T, Stake S, Miao S, Zuo YY, Harris BT, et al. Advances in 3D Bioprinting for Neural Tissue Engineering. Adv Biosyst. 2018;2(4):18. doi: 10.1002/adbi.201700213. PubMed PMID: WOS:000446969400003.
28. Pearse DD, Bunge MB. Designing cell- and gene-based regeneration strategies to repair the injured spinal cord. Journal of Neurotrauma. 2006;23(3-4):438-52. doi: 10.1089/neu.2006.23.437. PubMed PMID: WOS:000237337700016.
29. Teng YD, Lavik EB, Qu X, Park KI, Ourednik J, Zurakowski D, et al. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc Natl Acad Sci U S A. 2002;99(5):3024-9. Epub 2002/02/28. doi: 10.1073/pnas.052678899. PubMed PMID: 11867737; PubMed Central PMCID: PMCPMC122466.

30. Makadia HK, Siegel SJ. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers. 2011;3(3):1377-97. doi: 10.3390/polym3031377. PubMed PMID: WOS:000208601700026.
31. Fan J, Zhang H, He J, Xiao Z, Chen B, Xiaodan J, et al. Neural regrowth induced by PLGA nerve conduits and neurotrophin-3 in rats with complete spinal cord transection. J Biomed Mater Res B Appl Biomater. 2011;97(2):271-7. Epub 2011/03/07. doi: 10.1002/jbm.b.31810. PubMed PMID: 21384547.
32. Donaghue IE, Tator CH, Shoichet MS. Local Delivery of Neurotrophin-3 and Anti-NogoA Promotes Repair After Spinal Cord Injury. Tissue Eng Part A. 2016;22(9-10):733-41. doi: 10.1089/ten.tea.2015.0471. PubMed PMID: WOS:000377380600003.
33. Pritchard CD, Slotkin JR, Yu D, Dai H, Lawrence MS, Bronson RT, et al. Establishing a model spinal cord injury in the African green monkey for the preclinical evaluation of biodegradable polymer scaffolds seeded with human neural stem cells. J Neurosci Methods. 2010;188(2):258-69. Epub 2010/03/12. doi: 10.1016/j.jneumeth.2010.02.019. PubMed PMID: 20219534; PubMed Central PMCID: PMCPMC4157751.
34. Rauch MF, Hynes SR, Bertram J, Redmond A, Robinson R, Williams C, et al. Engineering angiogenesis following spinal cord injury: a coculture of neural progenitor and endothelial cells in a degradable polymer implant leads to an increase in vessel density and formation of the blood-spinal cord barrier. Eur J Neurosci. 2009;29(1):132-45. Epub 2009/01/06. doi: 10.1111/j.1460-9568.2008.06567.x. PubMed PMID: 19120441; PubMed Central PMCID: PMCPMC2764251.
35. Therapeutics I. Study of Probable Benefit of the Neuro-Spinal Scaffold™ in Subjects With Complete Thoracic AIS A Spinal Cord Injury as Compared to Standard of Care (INSPIRE 2) 2018 [updated November 22, 2021].
36. Courtine G, Bunge MB, Fawcett JW, Grossman RG, Kaas JH, Lemon R, et al. Can experiments in nonhuman primates expedite the translation of treatments for spinal cord injury in humans? Nat Med. 2007;13(5):561-6. doi: 10.1038/nm1595. PubMed PMID: 17479102; PubMed Central PMCID: PMCPMC3245971.
37. Rosenzweig ES, Brock JH, Lu P, Kumamaru H, Salegio EA, Kadoya K, et al. Restorative effects of human neural stem cell grafts on the primate spinal cord. Nat Med. 2018;24(4):484-+. doi: 10.1038/nm.4502. PubMed PMID: WOS:000429639800019.
38. Ko CC, Tu TH, Chen YT, Wu JC, Huang WC, Cheng H. Monkey Recovery from Spinal Cord Hemisection: Nerve Repair Strategies for Rhesus Macaques. World Neurosurg. 2019;129:e343-e51. Epub 2019/05/28. doi: 10.1016/j.wneu.2019.05.145. PubMed PMID: 31132502.
39. Sharif-Alhoseini M, Khormali M, Rezaei M, Safdarian M, Hajighadery A, Khalatbari MM, et al. Animal models of spinal cord injury: a systematic review. Spinal Cord. 2017;55(8):714-21. Epub 2017/01/24. doi: 10.1038/sc.2016.187. PubMed PMID: 28117332.
40. Emamhadi M, Soltani B, Babaei P, Mashhadinezhad H, Ghadarjani S. Influence of Sexuality in Functional Recovery after Spinal Cord Injury in Rats. Arch Bone Jt Surg-ABJS. 2016;4(1):56-9. PubMed PMID: WOS:000378681400011.
41. Batchelor PE, Skeers P, Antonic A, Wills TE, Howells DW, Macleod MR, et al. Systematic Review and Meta-Analysis of Therapeutic Hypothermia in Animal Models of Spinal Cord Injury. PLoS One. 2013;8(8):10. doi: 10.1371/journal.pone.0071317. PubMed PMID: WOS:000326473200040.
42. Macleod MR, McLean AL, Kyriakopoulou A, Serghiou S, de Wilde A, Sherratt N, et al. Risk of Bias in Reports of In Vivo Research: A Focus for Improvement. PLoS Biol. 2015;13(10):12. doi: 10.1371/journal.pbio.1002273. PubMed PMID: WOS:000364457500008.
43. du Sert NP, Bamsey I, Bate ST, Berdoy M, Clark RA, Cuthill IC, et al. The Experimental Design Assistant. Nat Methods. 2017;14(11):1024-5. Epub 2017/09/28. doi: 10.1038/nmeth.4462. PubMed PMID: 28960183.
44. Wang Q, Liao J, Hair K, Bannach-Brown A, Bahor Z, Currie GL, et al. Estimating the statistical performance of different approaches to meta-analysis of data from animal studies in identifying the impact of aspects of study design. bioRxiv. 2018:256776. doi: 10.1101/256776.
45. Gurevitch J, Koricheva J, Nakagawa S, Stewart G. Meta-analysis and the science of research synthesis. Nature. 2018;555(7695):175-82. Epub 2018/03/09. doi: 10.1038/nature25753. PubMed PMID: 29517004.

Figure legends

Figure 1 :Formats for biomaterials. A) A spinal cord injury with a large, irregularly shaped lesion site or cavity typical of a crush injury. This injury type is suited to injection of materials, including (counter clockwise, from upper right) a hydrogel loaded with microparticles, an amorphous hydrogel, a soft hydrogel, or a gel seeded with a defined cell type. B) A smaller, well defined injury site, more typical of a transection injury. This is suited to direct surgical insertion of scaffold materials, including (from top) fibrous materials with aligned or non-aligned matrices, a relatively firm hydrogel with or without a fibrous matrix, or a matrix with a porous character. The cavities in the material may form contiguous channels or be discontinuous. Created with icons from BioRender.com.

Figure 2: Flow diagram of included studies. Data from 134 publications were included in the meta-analysis and study quality/design assessment. Following data extraction, the analysis was conducted based on the set objectives. Of the included studies, 91 papers reported locomotor recovery outcomes, 72 reported *in vivo* axonal regeneration outcomes and 21 reported *in vitro* axonal regeneration. Objective 1 includes only comparisons that assessed the effect of biomaterials alone. Objective 2 includes studies that assessed BMC strategies *in vitro*, *in vivo*, and/or studied the biomaterial properties. Objective 3 includes studies that carried out investigations only *in vivo*.

Figure 3: Influence of the testing paradigm used on locomotor recovery outcomes and percentage of reporting study quality parameters.

(A) Percentage of studies reporting study quality parameters. (B) Effect of the influence of testing biomaterial properties and performing *in vitro* and *in vivo* experiments testing combinations (n = 29) *vs*. *in vivo* experiments only (n = 47) on the effect size as a percentage of improvement in motor score. Vertical error

bars represent the 95% CI for the individual estimates, and the horizontal shaded grey bar represents the 95% CI of the global estimate. The width of each vertical bar is normalised to the square root of number of animals contributing to that comparison.

Tables

Table 1: Objective 1, *meta-regression analysis of the effect of (A) the biomaterial format, (B) the specific biomaterial on locomotor recovery and (C) the specific biomaterial on in vivo axonal regeneration in BMO studies.*

A	***Improvement in locomotor outcomes***			
Biomaterial format	***Effect size (%)***	***P>|t|***	***95% Conf. Interval***	***Frequency % (n)***
Scaffold	10.4	0.001	[5.2, 15.6]	33.8 (23)
Microsphere-loaded hydrogel	9.6	0.967	[-3.8, 22.9]	8.8 (6)
Hydrogel (not injected)	8.9	0.695	[1.1, 16.7]	27.9 (19)
Linear oriented scaffold	4.6	0.189	[-4.2, 13.4]	19.1 (13)
Hydrogel (injected)	1.4	0.120	[-10.2, 13]	8.8 (6)
Other formats	8.7	0.907	[-20, 37.5]	1.5 (1)
	comparisons= 68, p=0.610, Tau^2= 88.43, I^2= 88.43%, adj R^2= 0%			
B	***Improvement in locomotor outcomes***			
Biomaterial name	***Effect size (%)***	***P>|t|***	***95% Conf. Interval***	***Frequency % (n)***
PHEMA-MMA	12	0.553	[-2.2, 26.2]	7.3 (5)
PLGA	8.7	0.875	[-3.8, 21.3]	8.7 (6)
Collagen	7.8	0.054	[-0.2, 15.7]	20.6 (14)
HA	6.6	0.863	[-7.1, 20.3]	7.4 (5)
Chitosan	4.7	0.578	[-6.3, 15.6]	11.8 (8)
HAMC-PLGA	-0.8	0.196	[-13.9, 12.3]	7.4 (5)
Other biomaterials	10.7	0.001	[1.3, 20]	36.8 (25)
	comparisons= 68, p=0.510, Tau^2= 78.4, I^2= 87.2%, adj R^2= 6.28%			

C	***Improvement in axonal regeneration***			
Biomaterial name	***Effect size (SD)***	***P>|t|***	***95% Conf. Interval***	***Frequency % (n)***
PLGA	0.9	0.901	[-0.6, 2.4]	9.5 (6)
Collagen	0.8	0.076	[-0.1, 1.6]	22 (14)
HA-PLGA	0.1	0.412	[-1.4, 1.7]	9.5 (6)
Other biomaterials	1.4	0.207	[0.4, 2.5]	59 (37)
	comparisons= 63, p=0.240, Tau^2=1.4, I^2=72%, adj R^2= 0.41%			

PHEMA-MMA: Poly(2-hydroxyethyl methacrylate-comethylmethacrylate), PLGA: Poly(lactic-co-glycolic-acid), HA: Hyaluronic acid, HAMC: hyaluronic acid methylcellulose.

Table 2: Objective 2, *meta-regression analysis of the effect of BMC strategies on (A) locomotor recovery and (B) in vivo axonal regeneration; combinations tested in vitro and/or in vivo.*

A	***Improvement in locomotor outcomes***			
Biomaterial-based combination	**Effect size (%)**	**P>|t|**	**95% Conf. Interval**	**Frequency % (n)**
PLGA + combinations	41.5	0.064	[16.7, 66.3]	4.9 (5)
Chitosan + combinations	27.3	0.289	[10.2, 44.4]	13.7 (14)
HA + combinations	22.5	0.684	[1.1, 43.9]	6.9 (7)
Collagen + combinations	18.1	0.002	[6.9, 29.2]	22.6 (23)
Fibrin + combinations	14.8	0.703	[-2.1, 31.7]	13.6 (14)
Other biom. + combinations	30.7	0.064	[17, 44.4]	37.9 (39)
	comparisons=102, p=0.142, Tau2=520.7, I2=97.7%, adj R2=4.11%			
B	***Improvement in axonal regeneration***			
Biomaterial-based combination	***Effect size (SD)***	***P>|t|***	***95% Conf. Interval***	***Frequency % (n)***
Chitosan + combinations	2.9	0.235	[0.9, 4.8]	6 (7)
PLGA + combinations	2.8	0.289	[0.7, 4.8]	5.1 (6)
LOCS + combinations	2.4	0.391	[0.7, 4.0]	6.8 (8)
Alginate + combinations	1.9	0.838	[-0.4, 4.1]	4.27 (5)
Collagen + combinations	1.7	0.001	[0.8, 2.5]	24 (28)
Matrigel + combinations	1.4	0.836	[-0.6, 3.5]	4.3 (5)
Fibrin + combinations	0.3	0.067	[-1.2, 1.8]	8.5 (10)
Fibrin-PLGA + combinations	-0.1	0.078	[-2, 1.8]	5.1 (6)
Other biom. + combinations	1.6	0.853	[0.5, 2.7]	35.9 (42)
	comparisons=117, p= 0.182, Tau^2=2.2, I^2=76.2%, adj R^2=9.3%			

PLGA: Poly(lactic-co-glycolic-acid), HA: Hyaluronic acid, LOCS: Linear ordered collagen scaffold.

Table 3: Objective 3, *meta-regression analysis of the effect of BMC strategies on (A) locomotor recovery and (B) in vivo axonal regeneration; combinations tested in vivo only.*

A	***Improvement in locomotor outcomes***			
Biomaterial-based combination	***Effect size (%)***	***P>|t|***	***95% Conf. Interval***	***Frequency % (n)***
PLGA + combinations	46.3	0.002	[26.4, 66.3]	12.8 (6)
Chitosan + combinations	19.8	0.476	[0.8, 38.8]	17 (8)
Collagen + combinations	13	0.055	[-0.3, 26.4]	27.7 (13)
Fibrin + combinations	11.3	0.845	[-67, 29.3]	19.1 (9)
Other biom. + combinations	40.1	0.004	[22.3, 57.8]	23.4 (11)
	comparisons=47, p=0.0006, Tau^2=318.4, I^2=95.9%, adj R^2=91.72%			
B	***Improvement in axonal regeneration***			
Biomaterial-based combination	***Effect size (SD)***	***P>|t|***	***95% Conf. interval***	***Frequency % (n)***
Chitosan + combinations	2.1	0.263	[-0.5, 4.8]	10.9 (5)
PLGA + combinations	1.7	0.348	[-0.5, 3.9]	19.6 (9)
Matrigel + combinations	1.5	0.53	[-1, 4]	10.9 (5)
Fibrin + combinations	0.7	0.326	[-0.7, 2]	21.7 (10)
Collagen + combinations	0.5	0.869	[-1.6, 2.6]	19.6 (9)
Other biom. + combinations	2.8	0.069	[0.5, 5.1]	17.4 (8)
	comparisons=46, p=0.398, Tau^2=2.56, I^2=88.5%, adj R^2=0%			

PLGA: Poly(lactic-co-glycolic-acid).

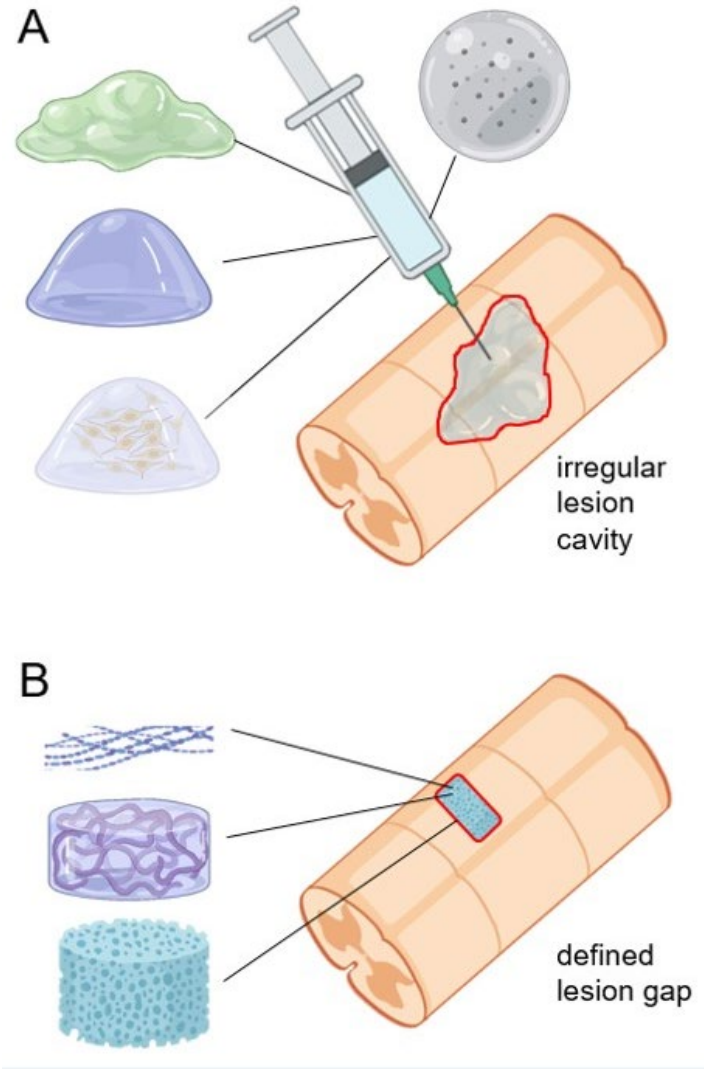

Figure 1

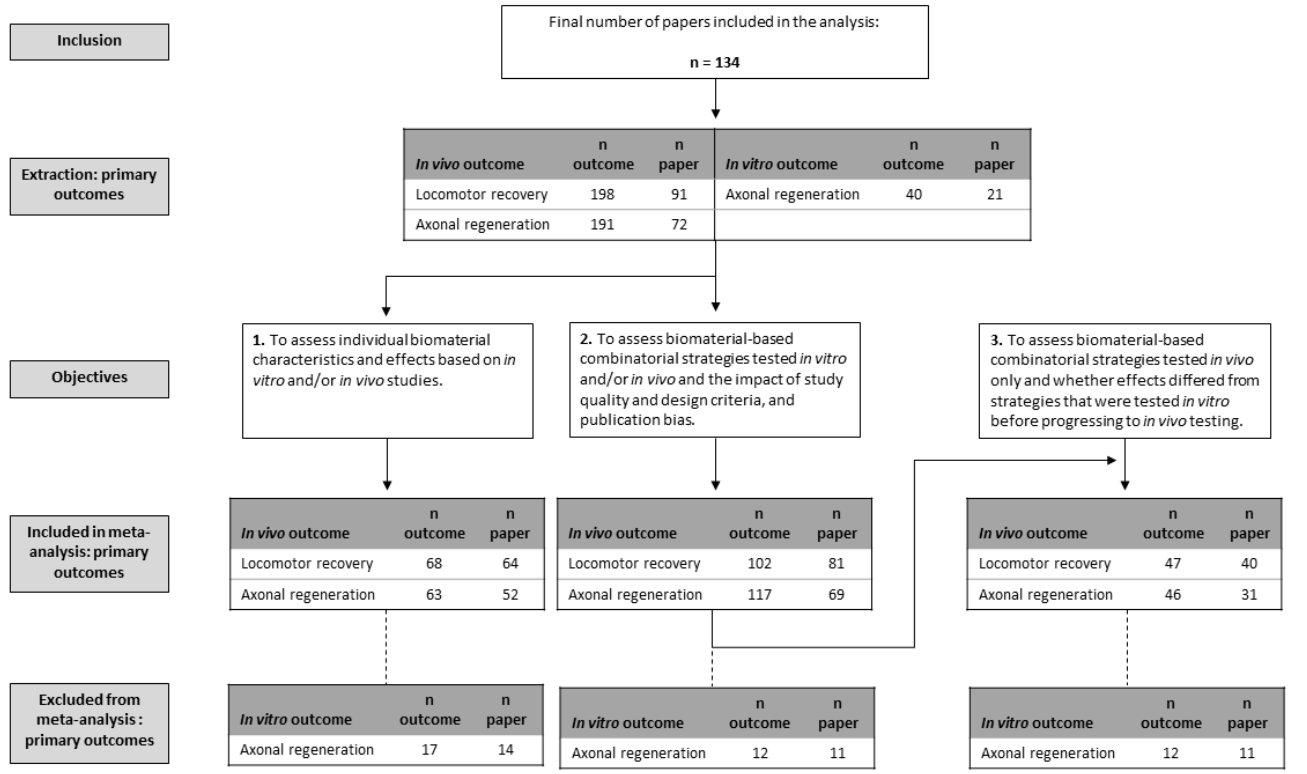

Figure 2

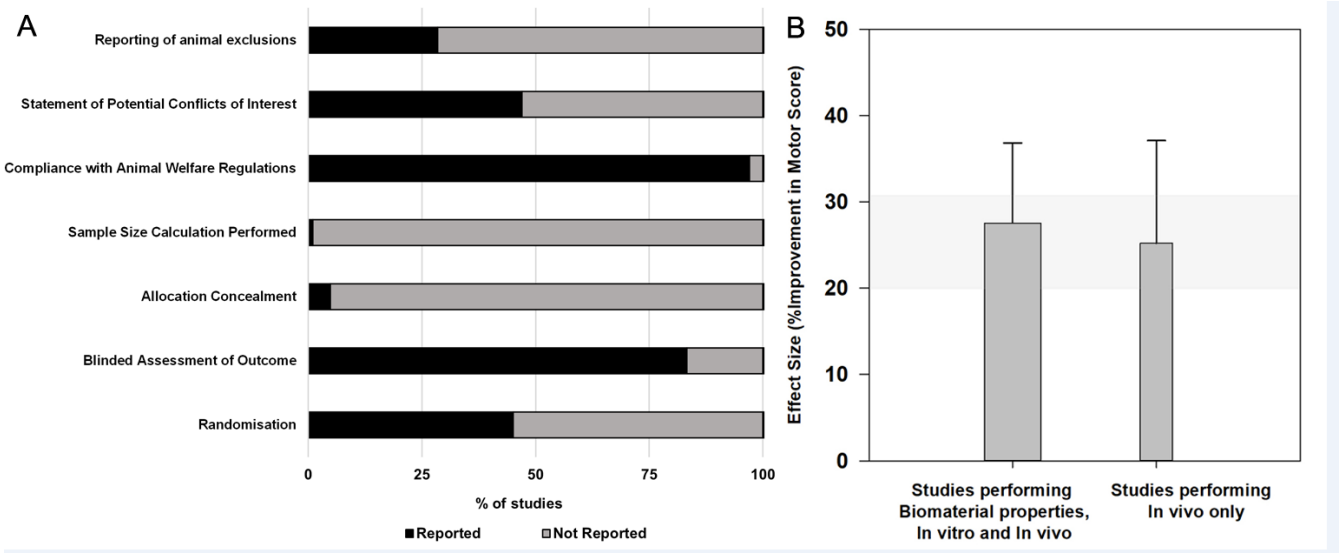

Figure 3